[0128] D. Post-Translational Modification

[0129] Protein function is often regulated by post-translational modifications such as the addition of sugar complexes, lipid anchors such as provided by myristoylation, geranyl-geranylation or famesylation, or by phosphorylation to mention a few. The regulation of protein function by phoshorylation or dephosphorylation is central in cell signal transduction.

[0130] Methods of the present invention can be used to study post-translational events or to identify phosphorylation sites. In a preferred embodiment, antibody fragments such as scFv are printed on Matrix-Assisted Laser Desportion/Ionization (MALDI) chips for detecting phosphorylation of known and suspected phosphorylation sites in proteins. Coupling proteins to reactive surface MALDI mass spectrometry surfaces was described in U.S. Pat. No. 6,020, 208, and incorporated herein by reference. The chip is commercially available from Ciphergen Biosystems Freemont, Calif. In an exemplary embodiment, phosphospecific antibodies against the apoptotic proteins Bcl-2, Bad, and caspase **9** are coupled to reactive surface MALDI chips, and are used for selective capture of phosphorylated fragments of these proteins. The chip can be analyzed for mass using time of flight mass spectrometry.

[0131] Methods of the present invention further provide a new way to detect the occurrence of a phosphorylation event on a known or unknown phospho-accepting residue using recombinant single chain antibodies (scFv) coupled with mass spectrometry. This method has been termed proximal phospho-affinity mapping, and serves as an alternative method that does not rely on the use of IMAC or the use of phospho-specific antibodies, which are notoriously difficult to make.

[0132] Referring to **FIG. 2**, an embodiment of this method uses recombinant single chain antibodies (scFv), polyclonal, or monoclonal antibodies **30** that are designed to recognize, instead of a phorsphorylation site **70** itself, an epitope **50** on the same antigen that is in proximity to the phosphorylation site **70**, whether site **70** is confirmed or just suspected for phosphorylation. The epitope **50** may be as close as 5-10 amino acids away, as long as the distance between the epitope **50** and the phosphorylation site **70** is such that antibody recognition is not hindered by a phorsphorylation event. Such an antibody or antibody fragment **30**, which is coupled to a support surface **10** through a linker **20**, will recognize the antigen **60** (e.g. a tryptic peptide) whether or not the antigen is phosphorylated. In an exemplary embodiment, peptides are generated using proteases such as trypsin or V8, or by non-enzymatic methods, such as CNBr. This yields peptide fragments that can be identified by their unique sizes. Among these fragments are the target fragments **60** that contains known or predicted phosphorylation sites. Single chain antibodies or traditional antibodies are panned or immunized against synthetic peptides that correspond to an epitope region **50** that is close to the phosphorylation site **70** in the tryptic fragment **60** using standard panning procedures. The epitope **50** may consist of as few as 3-7 amino acids. The antibody or antibody fragment that are generated may be used as capture molecule coupled to MALDI reactive chips. The chips may then be used to detect characteristic mass shift indicative of phosphorylation. Since this method enables parallel purification/identification and analysis of phosphorylation, it offers a valuable detection tool for phosphorylation screening. And because the antibody or antibody fragment generated according to this method recognizes the target peptide in both the phosphorylated and unphosphorylated state, this method is also useful in studying events and conditions that affect phosphorylation.

[0133] In a particularly preferred embodiment, the peptide **60** is selected in the following way: first, kinase substrate consensus sequences are located in the target protein through searches conducted in a database that contains protein sequence information. Then, a peptide containing such consensus sequence is selected through comparing the digestion maps of various proteases-peptides of about 20 amino acids are preferred. Last, an epitope other than the kinase substrate consensus sequences on the selected peptide is chosen for raising an antibody or antibody fragment.

[0134] E. Cellular Organelle

[0135] Methods of the invention can also be used to capture cellular organelles organelles from whole cell extracts or from fractions of whole cell extracts. In a preferred embodiment, an antibody that recognizes a voltage dependent anion channel ("VDAC") receptor uniquely associated with the mitochondrial membrane is printed as described earlier to capture Green Fluorescent-coupled cytochrome C expressing mitochondria. Dyes that have potentiometric quality can be used to specifically label mitochondria that have intact voltage gradient. The detection of captured mitochondria or other organelles from cells at different states can be used to indicate occurrence of apoptosis or other cellular events.

[0136] F. Others

[0137] Methods of the invention may also be used for other applications such as tissue typing, disease diagnosis, and evaluation of therapeutics. Biological samples from patients that may reveal genetic disorders (PCT patent publication No. 89/11548, incorporated herein by reference), may be used in the present invention. Likewise, this invention can be used to detect abnormality in protein expressions, the existence of antigens or toxins in a given sample. Further, methods of the invention can also be used to evaluate responses from organisms, tissues or individual cells to exposure to drugs, pharmaceutical lead compounds, or changes in environmental factors.

EXAMPLES

[0138] A. Substrate Surface Preparation

[0139] (i) Method of Stripping Glass Slide and Re-Packing with Reactive Groups

[0140] An example of this preferred method is as follows: first, a plain glass slide (VWR Scientific Products, for instance) is cleaned in a piranha solution (70:30 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 12 hours at room temperature. (Caution: "piranha" solution reacts violently with several organic materials and should be handled with extreme care). After thorough rinsing with water, the slides is treated with a silane solution, such as a **3**%solution of 3-aminopropyltriethoxysilane in 95% ethanol. And before treating the slides, the silane solution may be stirred for at least 10 minutes to allow hydrolysis and silanol formation.